In vivo calibration of a subcutaneous glucose sensor for determination of subcutaneous glucose kinetics

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G. Velho*, Ph. Froguel*, D.R. Thévenot** and G. Reach*

ABSTRACT. The aim of this work was to develop a method for calibrating *in vivo* a subcutaneous glucose sensor; this calibration procedure was used to investigate the kinetics of the estimated subcutaneous glucose concentration during variations in blood glucose level. These experiments were performed in anaesthetized rats: 1) In 25 animals, glycaemia was decreased from 5.8 ± 0.2 to a 2.9 ± 0.2 mmol/l plateau, following the intravenous injection of 1 unit of insulin. This drop was followed by a decrease in the signal amplitude with a 5 min time lag, from 13.54 ± 1.09 to 10.28 ± 1.05 nA ($p < 0.01$); 2) These two steady states allowed to perform a two-point *in vivo* calibration of the sensor and to calculate the apparent subcutaneous glucose level during subsequent glucose administration: a) Following a continuous glucose infusion (n = 6) that increased glycaemia within 35 min to a 5.6 ± 0.4 mmol/l plateau, the subcutaneous glucose level increased to a 5.1 ± 0.2 mmol/l plateau (NS); when glycaemia was increased to 11.3 ± 0.3 mmol/l (n = 7), subcutaneous glucose concentration raised to 10.7 ± 0.4 mmol/l (NS); b) In contrast, after a glucose bolus (n = 7), the glycaemic peak at 2 min was 12.4 ± 0.9 mmol/l, while the subcutaneous glucose peak, observed at 2 min, was only 6.2 ± 0.6 mmol/l ($p < 0.01$). We conclude, therefore, that under steady state conditions, the apparent subcutaneous glucose concentration reflects blood glucose level, thus validating the subcutaneous tissue as a site for the implantation of a glucose sensor to be used in a closed-loop insulin delivery system.

INTRODUCTION

Glucose sensors are an essential part of closed-loop insulin delivery systems in which an electric signal is generated by the sensor, as a function of glucose concentration, and is processed by a computer to regulate the flow rate of a pump delivering insulin (1). Most of the sensors proposed so far consist of a glucose electrode in which the oxidation of glucose by glucose-oxidase is responsible for the generation of the signal (2). In the past few years, needle type glucose sensors have been proposed for subcutaneous implantation (3-5). Indeed, a subcutaneous implantable sensor would be of great interest, since it could allow for easy replacement in case of impairment of the sensor performances. However, if a subcutaneous sensor is supposed to work as part of a closed-loop insulin delivery system, intended to regulate blood glucose (BG) level in diabetic patients, this assumes that the relationship between the generated current and BG concentration is well defined. As things now stand, the relationship between the true subcutaneous glucose concentration, which will be monitored, and the BG level is unknown, since the former is not measurable.

The aim of this work was, therefore, to characterize these relationships. As a first step, we defined a method for calibrating *in vivo* a subcutaneous glucose sensor, namely for determining, for a given sensor, the variation in the current corresponding to a change in BG concentration. This *in vivo* sensitivity coefficient was then used to determine from the sensor output an apparent subcutaneous glucose concentration and to estimate its variations occurring during modifications of BG concentrations. These experiments were performed in anaesthetized rats.

METHODS

Needle type glucose sensor

The design of the sensor was modified from Shichiri et al. (3). Briefly, the cathode was made of a steel needle (23G), coated with gold or silver by

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electrolysis (Usiflamme, Saint Gratien, France). The anode was formed by the tip of a platinum wire, melted by a gas flame to form a microsphere (1 mm diameter), then electrically isolated inside a polyethylene catheter (Biotrol, Paris, France) and cast in an epoxide resin inside the cathode needle. The electrode tip was then dipped into a suspension of 2000 IU/ml glucose oxidase (Grad II, Boehringer Mannheim, Mannheim, W. Germany) and 1000 IU/ml heparin (H-108, Choay, Paris, France) in acetone and ethanol (1:1 volume) containing 25% cellulose acetate (Prolabo, Paris, France). After air drying, it was dipped into a 1% glutaraldehyde hydric solution (Prolabo), and again allowed to air dry. It was then dipped in a solution of 5% polyurethane (EG85A, Thirmedics, Woburn Mass., U.S.A.) in dimethylformamide and tetrahydrofuran (1:9 volume, Prolabo). Forty to 50% of the prepared sensors had satisfactory *in vitro* linearity range (higher than 15 mmol/l) and response time ($t_{90\%}$ shorter than 1 min). Other sensors were discarded. Sensors were stored dry at 4° C, between preparation and implantation (3 to 10 days). Each experiment (n = 25) was performed with a different sensor, to make sure that the calibration results were independent of the characteristics of a given sensor.

In vitro calibration of the sensor

The sensor was connected to an amperometric unit (PRG-Del, Tacussel Electronique, Villeurbanne, France) imposing a 650 mV voltage difference between the anode and the cathode and allowing for current recording (pen recorder SE120, Goerz Electro, Vienna, Austria), expressed in nanoamperes. Before each *in vivo* experiment, the characteristics of the sensor (i.e. the *in vitro* sensitivity, expressed in nA/mmol/l glucose, the linearity range and the response time) were determined in physiological saline solution by recording the basal sensor current (I_0) for 30-60 min (until signal stabilization), and the sensor response to stepwise increases in the glucose concentration. This *in vitro* study was repeated after explantation of the sensor.

In vivo calibration: determination of the in vivo sensitivity coefficient

Each experiment was carried out with a different animal (n = 25). Overnight fasted male Wistar rats (250-300 g body weight, Janvier, Saint Berthevi, France) were anaesthetized with pentobarbital (50 mg/kg intraperitoneally, Clin Midy, Saint Jean de la Ruelle, France), and polyethylene (Biotrol 3, Biotrol, France) and silicone (Silastic 602-135, Dow Corning, Midland Mich, U.S.A.) catheters were inserted respectively into the left jugular vein and homolateral carotide artery, for glucose or insulin injection, and for blood sampling for the determination of BG concentration. After the *in vitro* characterization, the sensor was implanted in the subscapular subcutaneous tissue by blunt dissection. Animals were kept spontaneously breathing under a flow of carbogen (O_2:CO_2, 95:5) and warmed under a lamp.

At least 1 hr after surgery, when BG and the sensor output were stable, 1 U of insulin (Endopancrine U40, Organon, Saint Denis, France) was injected through the jugular catheter. Blood samples (0.3 ml) were serially drawn through the carotid catheter, from —10 to 50 min, with a 5 min interval. Samples were immediately centrifuged and plasma glucose concentration was determined with a glucose analyzer (Beckman, Fullerton Ca., U.S.A.). Following insulin injection, the sensor output decreased to reach a plateau, as did the BG concentration. The pre-insulin and post-insulin steady states of the BG level and of the sensor output made it possible to calculate, by a two-point calibration of the sensor, an *in vivo* sensitivity coefficient, expressed in nA/mmol/l, as the ratio between the decrease in the sensor current and the decrease in glucose concentration, and an extrapolated *in vivo* residual current (I_0) which would be observed in the absence of glucose.

In the experiments where glucose was continuously administered after the insulin-induced hypoglycaemia (see below), a second plateau was reached both for the BG concentration and the sensor signal. It was therefore possible to calibrate the sensor a second time and to calculate, under these new conditions, a second *in vivo* sensitivity coefficient based on these pre and post-glucose infusion plateaus.

Determination of subcutaneous glucose concentration

The *in vivo* sensitivity coefficient, determined in each individual experiment was used to calculate the apparent subcutaneous glucose level. This estimation of subcutaneous glucose was obtained by substracting from the sensor current, observed at a

given time, the *in vivo* residual current (I_0), and then by dividing the resultant current by the sensitivity coefficient. This calculation was carried out in three situations: the insulin-induced hypoglycaemia itself and the subsequent i.v. glucose administrations, either as a continuous glucose infusion (20 to 60 mg/kg/min) using an infusion pump (Infu 362, Datex, Uhwiesen, Switzerland), or as a glucose bolus (0.5 g/kg), These glucose administrations were performed shortly after a stable hypoglycaemic level was achieved.

Expression of results and statistics

All data in text and figures are given as mean ± SEM, and their statistical significance was assessed by the two-tail paired t-test.

RESULTS

In vitro characteristics of the sensors

When assessed *in vitro*, respectively before implantation and after explantation, the linearity range of the sensor response was up to 18.0 ± 0.9 and 15.3 ± 0.9 mmol/l of glucose (n = 25), and its response time ($t_{90\%}$) to each glucose increment was 44 ± 4 and 42 ± 2 seconds. The *in vitro* sensitivity of the sensor, was 0.82 ± 0.10 and 0.92 ± 0.1 nA/mmol/l and the current observed in the absence of glucose (I_0) 8.04 ± 1.05 and 7.73 ± 1.18 nA, before and after the *in vivo* experiment, respectively.

In vivo calibration

Following the i.v. injection of 1 U of insulin (Fig. 1), BG level decreased from 5.8 ± 0.2 to a 2.9 ± 0.2 mmol/l plateau and the current generated by the sensor decreased, with a five min time lag, from 13.54 ± 1.09 to 10.28 ± 1.05 nA (n = 25, $p < 0.01$). The high amplitudes of the SEM reflect the variability of the 25 different sensors' sensitivity coefficient. The *in vivo* sensitivity coefficient calculated, in each experiment, by this procedure was 1.15 ± 0.10 nA/mmol/l glucose ($p < 0.05$ versus the *in vitro* value). The extrapolated signal output for 0 mmol/l glucose concentration (I_0) was 7.00 ± 1.13 nA (N.S. versus the *in vitro* values). The correlation coefficient for the signal amplitude (nA) vs BG level (mmol/l) was in each experiment greater than 0.96.

Estimation of the subcutaneous glucose concentration

Figure 2 represents the change in subcutaneous glucose concentration, during insulin-induced hypoglycaemia, calculated from the sensor output (Fig. 1) and taking into account in each experiment the respective *in vivo* sensitivity coefficient. The curve reflects the changes in BG levels with 5-min delay. One must note that after the transformation of the data, the variance of the estimated subcutaneous glucose concentration was, at any time, similar to that of BG. In 6 of these experiments, a 20 mg/kg/min glucose infusion was initiated shortly after the insulin-induced hypogly-

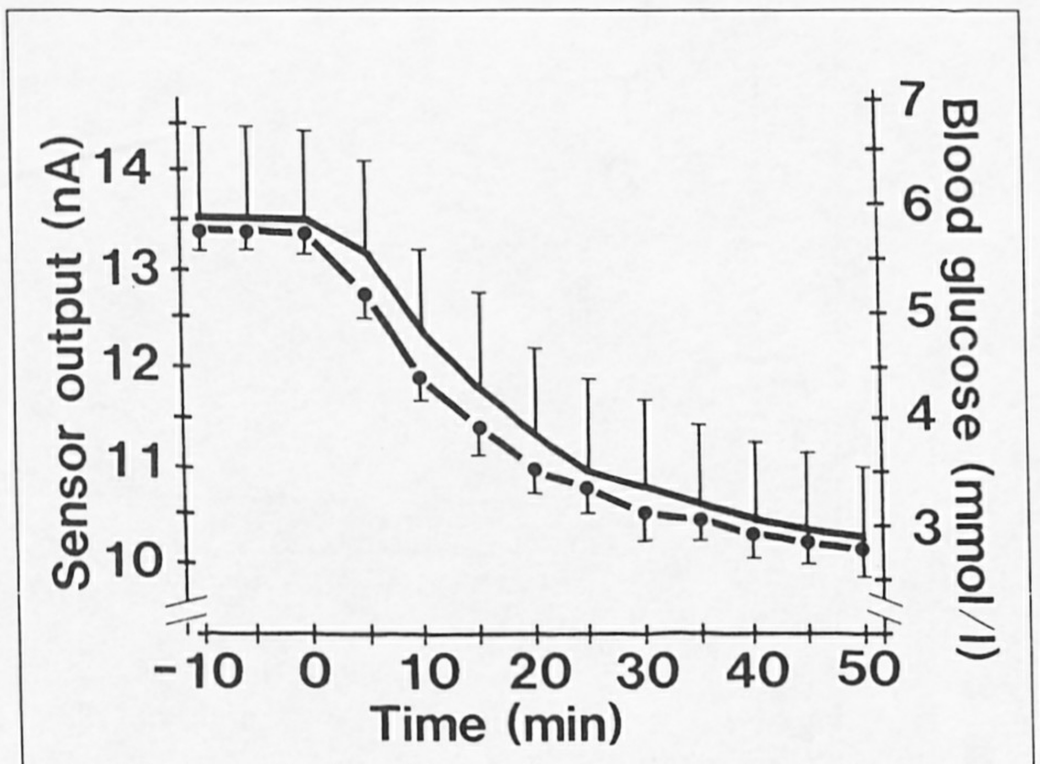

Fig. 1 - *Plasma glucose concentration (closed circles) and sensor output (solid line) following the injection of 1 U of insulin at time zero (mean ± SEM, n = 25).*

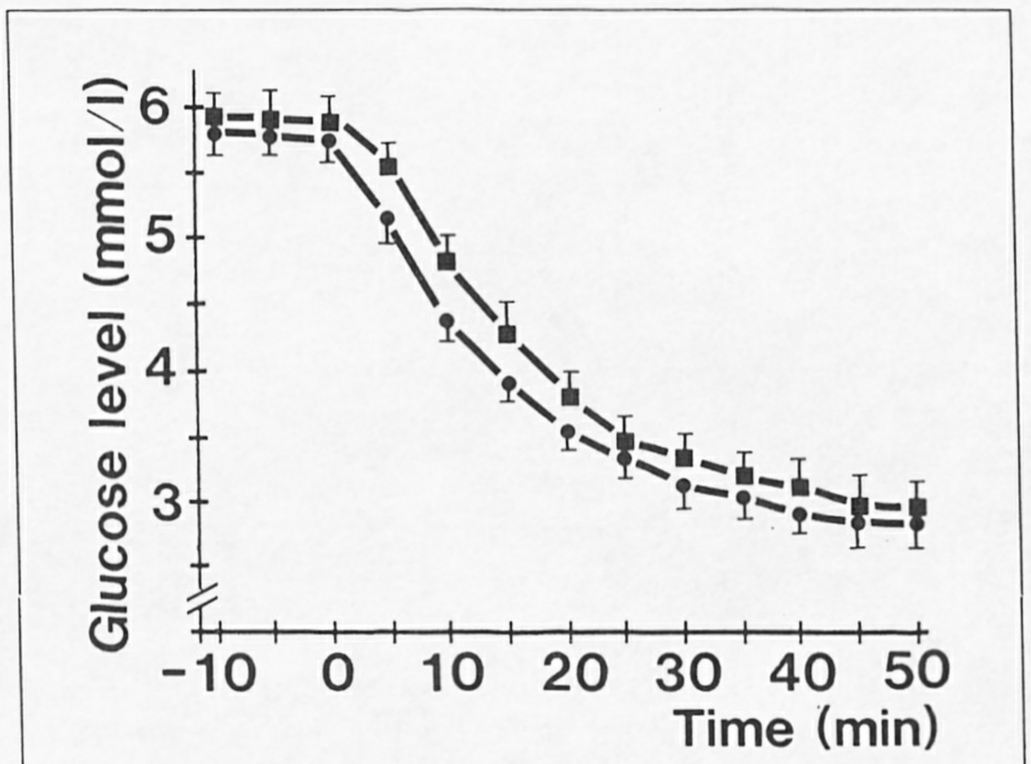

Fig. 2 - *Plasma glucose concentration (closed circles), and apparent subcutaneous glucose level (closed squares) calculated from Figure 1, following insulin injection (mean ± SEM, n = 25).*

caemic plateau was reached (Fig. 3). It increased the BG concentration from 3.5 ± 0.5 mmol/l to a 5.6 ± 0.4 mmol/l plateau. In the meantime, the estimated subcutaneous glucose level rose to a 5.1 ± 0.2 mmol/l plateau (NS). In 7 other experiments (Fig. 3), glucose infusion rate was progressively increased from 20 to 60 mg/kg.min^{-1} to reach a higher BG concentration. Blood glucose increased progressively from 2.5 ± 0.2 to 11.3 ± 0.3 mmol/l and the estimated subcutaneous glucose concentration increased, with a 5 min time lag, to reach 10.7 ± 0.4 mmol/l at 40 min (NS). Figure 4 represents the correlation between the estimated subcutaneous glucose concentrations and BG levels reached at the different plateaus obtained during these series of experiments (21 values obtained with 7 different sensors, which are represented by different symbols). As can be seen, for each sensor, these two parameters were linearly correlated, the mean value of the "r" coefficient being 0.9987 ± 0.0002 (n = 7). This figure was obtained by using only stationary levels of BG and sensor current observed before and after insulin or glucose infusions, with at least 5 min of stable levels, thus overcoming the bias which would be introduced by the time lag between changes in blood and subcutaneous glucose concentrations.

After a 0.5 mg/kg glucose bolus injection (n = 7, Fig. 5), the peak in BG concentration, at 2 min, was 12.4 ± 0.9 mmol/l, while the apparent subcutaneous glucose peak, also observed at 2 min, was only 6.2 ± 0.6 mmol/l ($p < 0.01$). Both the BG concentration and the estimated subcutaneous glucose level returned to basal at 30 min (3.1 ± 0.7 mmol/l vs 2.2 ± 0.4 mmol/l, respectively, N.S.).

Comparison of two calibrations of the sensor

The inverse of the *in vivo* sensitivity coefficient (1/x) is the parameter which describes the variation in the subcutaneous glucose concentration responsible for a one nA variation in the sensor output, and can therefore be used in the algorithms of the insulin infusion control system. This value was identical when calculated on the basis of the plateaus reached before and after the insulin injection (1.17 ± 0.19 mmol.l^{-1}/nA), or of those observed before and after the glucose infusions (1.21 ± 0.18 mmol/l/nA, n = 13, NS). The correlation between these two values was r = 0.998 (y = 0.97x + 0.087 mmol/l/nA). Similarly the two estimations of I_0 were identical (5.69 ± 1.46 nA vs. 5.53 ± 1.53 nA, n = 13, NS) and well correlated (r = 0.977, y = 1.04x — 0.38).

DISCUSSION

This work was undertaken to define a method of calibration of a glucose sensor implanted in the

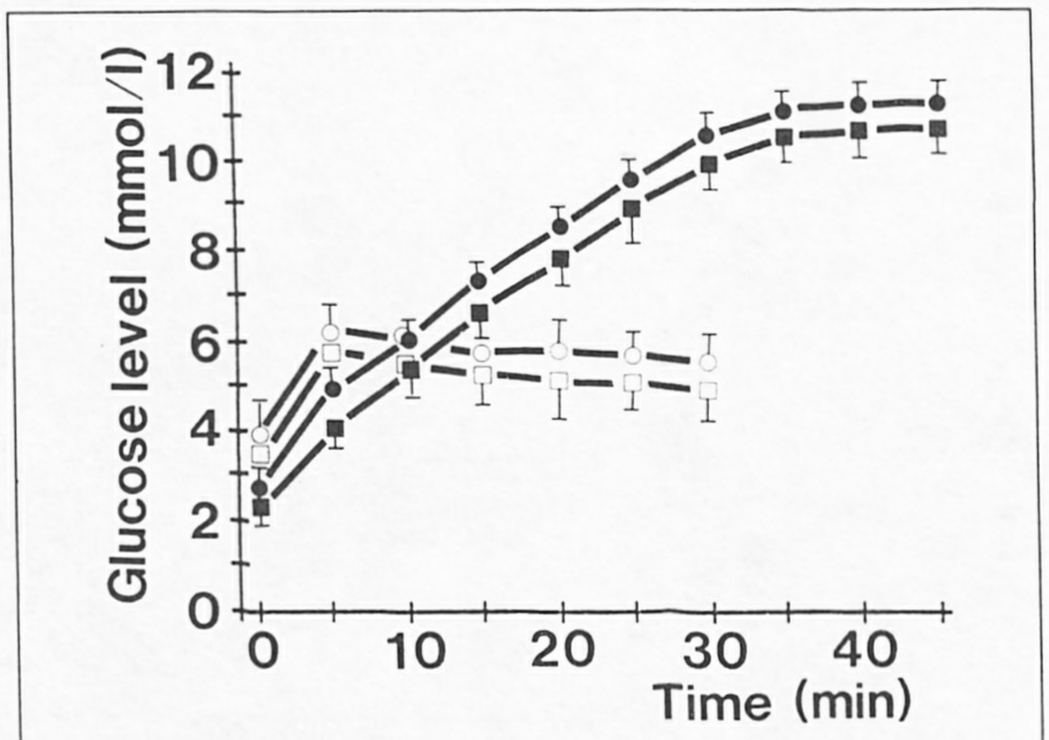

Fig. 3 - *Plasma glucose concentration (circles) and apparent subcutaneous glucose level (squares) during intravenous glucose infusion: A) 20 mg/kg/min, open symbols, n = 6; B) 20-60 mg/kg/min, closed symbols, n = 7. Glucose infusion was initiated when a stable hypoglycaemic level was achieved following the insulin injection (Fig. 2). Mean ± SEM.*

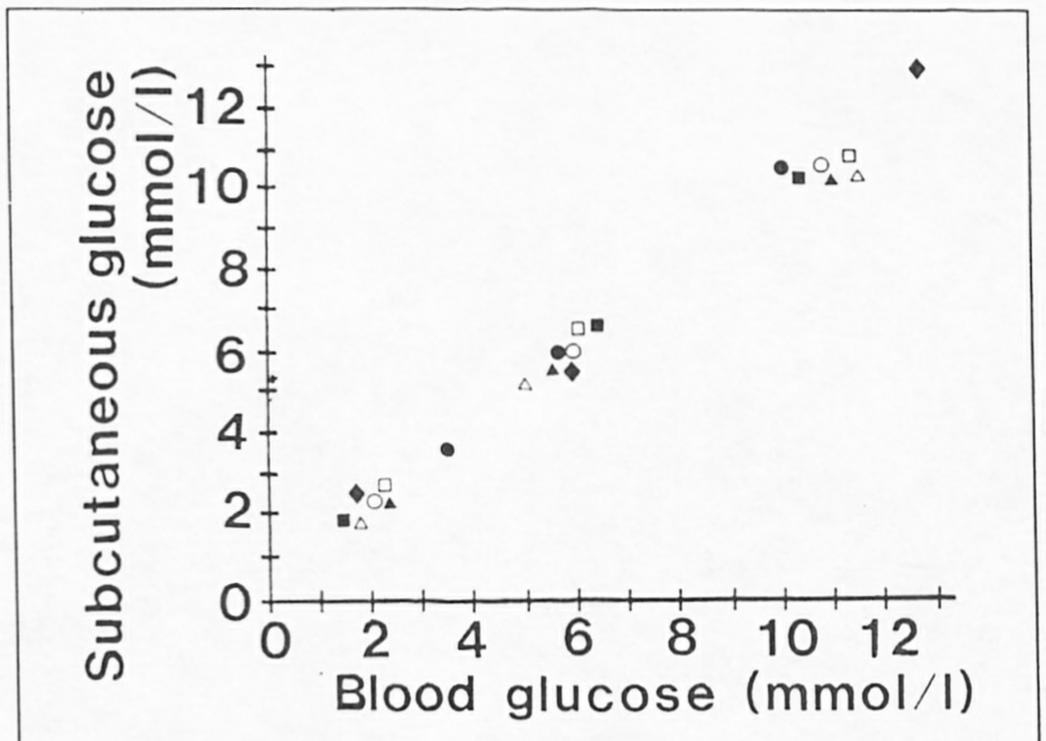

Fig. 4 - *Correlation between the estimation of subcutaneous glucose concentration and the concomitant plasma glucose level reached at low, basal and high glucose level plateaus obtained during the experiments of insulin induced hypoglycaemia and glucose infusion. Each symbol represents a different sensor (21 points for 7 sensors, r = 0.9987 ± 0.0002, n = 7, Mean ± SEM).*

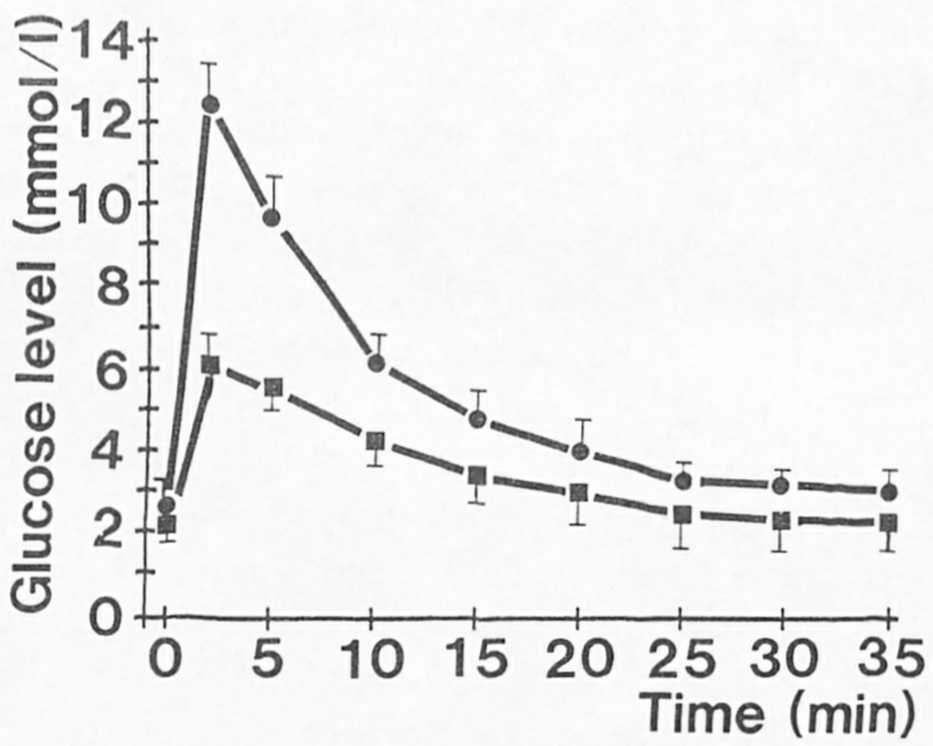

Fig. 5 - *Plasma glucose concentration (closed circles) and apparent subcutaneous glucose level (closed squares) following i.v. glucose injection (0.5 g/kg), administered when a stable hypoglycaemic level was achieved following the insulin injection (Fig. 2). Mean ± SEM of 7 experiments.*

subcutaneous tissue. This issue is critical since this calibration will allow us to calculate, from the sensor output, an estimation of the prevailing subcutaneous glucose concentration. This estimation, processed by means of algorithms, will determine the flow rate of the pump delivering insulin, in a closed-loop insulin delivery system. However, the calibration of a subcutaneous implanted sensor presents a specific difficulty, which is due to the fact that the relationship between the true subcutaneous glucose concentration and the BG level is unknown. For these reasons, Matthews et al. (5) and Pfeiffer et al. (6) preferred to present the sensor output expressed in nA, and not to propose any transformation into an apparent glucose concentration.

Shichiri et al. (3), and Claremont et al. (4) calibrated the glucose sensor on the basis of an *in vitro* calibration, performed respectively in physiological saline, or in a buffered glucose solution. This assumes, however, that both the current observed in the absence of glucose (I_0) and the sensitivity of the sensor are identical under both *in vitro* and *in vivo* conditions. This might not be true, and might be one explanation for the very low values of the estimated glucose concentration in pig subcutaneous tissue, which was only 20% of the concomitant BG level (4). If we consider our experiments in rats, the *in vitro* and *in vivo* values of I_0 obtained in each experiment were correlated ($r = 0.479$, $p < 0.05$), but we did not observe any correlation between the values of the sensitivity coefficient, determined under both conditions, ($r = 0.146$, $n = 25$), which were found to be significantly different ($p < 0.05$), with an unexpected higher value observed *in vivo;* although we do not have an explanation for this phenomenon, the absence of correlation between *in vitro* and *in vivo* data suggests that it might be hazardous to speculate on a difference observed between data obtained under two completely different situations. In addition, when some sensors were tested *in vitro* in two different buffers (data not shown), the observed sensitivity coefficients were well correlated ($r = 0.98$) but no correlation was found between the observed values of I_0 ($r = 0.668$, $n = 5$). Claremont et al. tested their sensor *in vitro* in a buffered glucose solution or in plasma; they found similar values for I_0, but a significantly lower value for the sensitivity coefficient when the sensor was tested in plasma (7). This suggests that the *in vitro* characteristics of a glucose sensor might depend on the choice of the buffer, which is in any way different from the interstitial fluid present in the subcutaneous tissue.

Abel et al. calibrated a glucose sensor implanted in dogs, by adjusting the initial sensor output to the BG concentration, and this *in vivo* calibration procedure was used to determine the subcutaneous glucose concentration during a subsequent glucose infusion (8). However, this is a one-point calibration procedure, and it assumes that the *in vivo* value of I_0 was identical to the *in vitro* value. If we consider the data obtained with our sensors, both values, as mentioned above were fairly correlated. It seems that this might have been the case with their sensors, since in their experiments in dogs, the estimated value of subcutaneous glucose concentration remained very close to BG level during a glucose infusion, in accordance to our data in rats.

To avoid these problems, we used an original *in vivo* two-point calibration procedure, which made it possible to transform, in each individual experiment, the recorded current into an estimation of the subcutaneous glucose concentration, without having to take into account the *in vitro* data. This method allowed us to determine all the *in vivo* characteristics of the sensor: sensitivity coeffi-

cient, value of I_0, and even, for some sensors, the linearity range (Fig. 4). This figure is most important since it demonstrates that for each individual sensor the apparent subcutaneous glucose concentration, estimated with this method (and therefore the sensor output from which it was calculated), was linearly correlated to the corresponding BG level, up to 13 mmol/l, although the sensor *in vivo* calibration itself was performed at a much lower level (between 6 and 3 mmol/l, Fig. 1). This validates *a posteriori* the calibration procedure which assumes that this relationship is linear. Furthermore, this calibration can be carried out under both upward and downward changes in BG concentrations, provided that two steady levels of BG are achieved. This might have practical implications for the clinical use of a subcutaneous glucose sensor, since obviously it would be safer to use a calibration procedure based on an increase in BG concentration: for instance, a two-point calibration procedure could be achieved by using the data of two successive BG determinations, performed by the patient before and after breakfast. Incidentally, the fact that these two estimations of the *in vivo* sensitivity coefficient were identical, as were the two estimations of this coefficient performed *in vitro*, before and after the *in vivo* experiment, suggest that the characteristics of the sensor did not change over the duration of the experiment (4 hr). Finally, note that the upper limit of the *in vitro* linearity range of the sensors was 18 mmol/l glucose, and that the *in vivo* linearity range of the sensors might actually exceed 13 mmol/l (which was the plateau value used in these experiments). We used this method of calibration to determine the *in vivo* kinetics of the apparent subcutaneous glucose concentration during glycaemic variations. These kinetics are of paramount importance for the design of the algorithms of a closed-loop insulin delivery system. Our data in rats demonstrate that, 1. during insulin induced hypoglycaemia and glucose infusion, the apparent subcutaneous glucose concentration follows BG with a 5 min delay, which represented the integration of the sensor response time and of glucose transfer time from blood to subcutaneous fluid compartment. Abel et al. observed a slightly longer delay in dogs (8), which might be due to the slower response time of their sensor, or to some difference between rat and dog subcutaneous tissues: 2. Under steady state conditions, the apparent subcutaneous glucose concentration is linearly related to that of BG (Fig. 4), up to 13 mmol/l: 3. This might not be true during an acute and transient change in BG concentration, achieved during an i.v. glucose injection. Although the peak BG concentration (12.4 mmol/l) remained in the *in vivo* linearity range of the sensor, the peak in the apparent subcutaneous glucose concentration was much less pronounced. Claremont also observed a difference between blood and subcutaneous glucose concentration peaks following a glucose bolus (4). This difference was probably due to the response time of the sensor, to the glucose transfer time from blood to subcutaneous tissue, and to the dilution of glucose in the extracellular space, where it was monitored by the sensor, the glycaemic peak being higher than the subcutaneous peak because it was measured before the intravascular and the extravascular glucose concentrations had reached an equilibrium.

It has to be pointed out that we did not measure the true subcutaneous glucose concentration, but a function of the sensor current, which was referred to throughout this text as an "apparent" subcutaneous glucose concentration. Nevertheless, two different approaches of sampling interstitial fluid in dogs, found that, under stationary conditions, the true subcutaneous glucose concentration is indeed identical to BG level: Fischer et al., by a wick technique, using subcutaneously implanted cotton threads (9), and Janle-Swain et al. using a subcutaneously implanted capillary filtrate collector (10). These data contrast with those obtained by Claremont in pigs (4) and Shichiri in dogs (3) and in human diabetic patients (11), who found lower values in the subcutaneous tissue. These underestimations may have been due to the use, in these later studies, of *in vitro* determined sensitivity coefficients to calculate the subcutaneous glucose concentration.

In conclusion, we described an *in vivo*, rather than an *in vitro* method for calibrating a subcutaneous glucose sensor; this method made it possible to characterize the relationship between BG and apparent subcutaneous levels, which were found to be virtually identical during progressive glycaemic changes, with a 5 min time lapse. Moreover, the fact that these relationships were highly reproducible (Figs. 2 and 3) demonstrates that this pa-

rameter is suitable to be processed in a closed-loop insulin delivery system using appropriate algorithms. Thus, if these data, obtained in rats, are relevant to man, they would validate subcutaneous tissue as a site for the implantation of a glucose sensor, as part of an artificial endocrine pancreas.

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